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L1: Entry 1 of 1 File: USPT Mar 2, 1999

US-PAT-NO: 5877402
DOCUMENT-IDENTIFIER: US 5877402 A

TITLE: DNA constructs and methods for stably transforming plastids of multicellular plants and expressing recombinant proteins therein

DATE-ISSUED: March 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Maliga; Zora Svab	East Brunswick	NJ		
Staub; Jeffrey M.	Marlton	NJ		
Zoubenko; Oleg	Piscataway	NJ		
Allison; Lori A.	Highland Park	NJ		
Carrer; Helaine	Piracicaba			BRX
Kanevski; Ivan	Piscataway	NJ		

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APPL-NO: 8/ 189256 [PALM]
DATE FILED: January 31, 1994

PARENT-CASE:
This application is a continuation-in-part of U.S. Ser. No. 08/111,398, filed Aug. 25, 1993, now U.S. Pat. No. 5,451,513 which is a continuation of U.S. Ser. No. 07/518,763, filed May 1, 1990, now abandoned.

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PRIOR-ART-DISCLOSED:

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0251654	January 1988	EPX	

OTHER PUBLICATIONS

Aldrich et al., Curr. Genet., 14: 137-46, (1988).
Barkan, EMBO J., 7: 2637-44, (1988).

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CLAIMS:

What is claimed is:

1. A DNA construct for stably transforming plastids of multicellular plants, which comprises a transforming DNA having:

a) a targeting segment comprising a DNA sequence homologous to a pre-determined plastid genomic sequence of a genome within a plastid to be transformed, said targeting segment enabling insertion of said transforming DNA into said plastid genome by homologous recombination with said pre-determined plastid genomic sequence, said insertion not interfering with the normal function of the plastid genome;

b) a chimeric selectable marker gene disposed within said targeting segment, positioned so as not to disrupt said homologous recombination, said chimeric selectable marker gene comprising:

i) a selectable marker coding segment encoding a gene product that confers to cells of said plant a selectable phenotype in the presence of a selection agent which is non-lethal to plant cells containing plastids transformed with said DNA construct but lethal to untransformed plastids;

ii) a 5' regulatory segment of plant chloroplast origin, operably linked to said selectable marker coding segment to promote expression of said selectable marker coding segment in said plastids; and

iii) a 3' regulatory segment of a plant chloroplast mRNA molecule, operably linked to said selectable marker coding segment to promote stability of mRNA produced during said expression of said selectable marker coding segment in said plastids; and

c) at least one cloning site adapted for insertion of at least one additional DNA segment, said at least one cloning site being disposed within said targeting segment so as not to interfere with said conferring of said selectable phenotype to said plant

cells.

2. A vector comprising a DNA construct according to claim 1.

3. A DNA construct for stably transforming plastids of multicellular plants and expressing at least one gene product within said transformed plastids, which comprises a transforming DNA having:

a) a targeting segment comprising a DNA sequence homologous to a pre-determined plastid genomic sequence of a genome within a plastid to be transformed, said targeting segment enabling insertion of said transforming DNA into said plastid genome by homologous recombination with said pre-determined plastid genomic sequence, said insertion not interfering with the normal function of the plastid genome;

b) a selectable marker gene disposed within said targeting segment, said selectable marker gene conferring to cells of said plant a selectable phenotype in the presence of a selection agent which is non-lethal to plant cells containing plastids transformed with said DNA construct but lethal to untransformed plastids; and

c) at least one additional expressible DNA segment disposed within said targeting segment, positioned so as not to disrupt said homologous recombination, and so as not to interfere with said conferring of said selectable phenotype to said plant cells, said at least one additional expressible DNA segment containing;

i) at least one coding segment encoding a gene product;

ii) a 5' regulatory segment of plant chloroplast origin, operably linked to said at least one coding segment to promote expression of said coding segment in said plastids; and

iii) a 3' regulatory segment of a plant chloroplast mRNA molecule operably linked to said at least one coding segment to promote stability of mRNA produced during said expression of said coding segment in said plastids.

4. A vector comprising a DNA construct according to claim 3.

5. A DNA construct as claimed in claim 1, wherein said pre-determined plastid genomic sequence is located in a large single-copy region of said genome.

6. A DNA construct as claimed in claim 5, wherein said pre-determined plastid genomic sequence is a sequence located between an rbcL gene and an accD gene in said large single-copy region.

7. A DNA construct as claimed in claim 5, wherein said pre-determined plastid genomic sequence is a sequence located between genes and operons in said large single-copy region selected from the group consisting of: psbE operon and petA operon, trnG gene and trnfM gene, and psbA gene and trnH gene.

8. A DNA construct as claimed in claim 1, wherein said pre-determined plastid genomic sequence is located in an inverted repeat region of said genome.

9. A DNA construct as claimed in claim 8, wherein said pre-determined plastid genomic sequence is a sequence located between a trnV gene and a 16S rRNA gene in said inverted repeat region.

10. A DNA construct as claimed in claim 8, wherein said pre-determined plastid genomic sequence is a sequence located between a trnV gene and an rps7/12 operon in said inverted repeat region.

11. A DNA construct as claimed in claim 1, wherein said pre-determined plastid genomic sequence is selected such that transcription of said transforming DNA occurs substantially without read-through transcription.

12. A DNA construct as claimed in claim 11, wherein said pre-determined plastid genomic sequence is a sequence located between divergently-transcribed genes.

13. A DNA construct as claimed in claim 12, wherein said pre-determined plastid genomic sequence is a sequence located between a trnV gene and an rps12/7 operon in said genome.

14. A DNA construct as claimed in claim 11, wherein said pre-determined plastid genomic sequence is a sequence located between adjacent tRNA genes in said genome.

15. A DNA construct as claimed in claim 1, wherein said targeting segment comprises SEQ ID NO: 3.

16. A DNA construct as claimed in claim 1, wherein said 5' regulatory segment of said chimeric selectable marker gene comprises a promoter and a translational control region, said promoter being positioned immediately upstream from said translational control region, said translational control region comprising a DNA sequence that encodes a ribosome binding site and a translation start codon.

17. A DNA construct as claimed in claim 16, wherein said translational control region of said 5' regulatory segment further comprises a DNA sequence that encodes at least one codon immediately downstream from said translation start codon.

18. A DNA construct as claimed in claim 1, wherein said non-lethal selectable phenotype comprises resistance to an antibiotic and said selectable marker coding segment of said chimeric selectable marker gene encodes a gene product that inactivates said antibiotic.

19. A DNA construct as claimed in claim 18, wherein said selectable marker coding segment encodes aminoglycoside adenyltransferase.

20. A DNA construct as claimed in claim 19, wherein said selectable marker coding segment comprises an aadA gene coding region.

21. A DNA construct as claimed in claim 19, wherein said chimeric selectable marker gene comprises a sequence selected from the group of sequences consisting of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11.

22. A DNA construct as claimed in claim 20, which is disposed within a vector to form plasmid pZS197.

23. A vector comprising a DNA construct as claimed in claim 2, wherein said DNA construct is disposed within a plasmid vector to form plasmids selected from the group consisting of pPRV1, pPRV100, pPRV111, pPRV112, pPRV121, pPRV211, pZS208, and pZS209.

24. A DNA construct as claimed in claim 3, wherein said 5' regulatory segment of said expressible DNA segment comprises a promoter and a translational control region, said promoter being positioned immediately upstream from said translational control region, said translational control region comprising a DNA sequence that encodes a ribosome binding site and a translation start codon.

25. A DNA construct as claimed in claim 24, wherein said translational control region of said 5' regulatory segment further comprises a DNA sequence that encodes at least one codon immediately downstream from said translation start codon.

26. A DNA construct as claimed in claim 24, wherein said promoter is light-inducible.

27. A DNA construct as claimed in claim 24, wherein said promoter is tissue-specific.

28. A DNA construct as claimed in claim 3, wherein said selectable marker gene is a chimeric gene comprising:

i) a selectable marker coding segment encoding a gene product that confers a non-lethal selectable phenotype to cells containing plastids transformed with said DNA construct;

ii) a 5' regulatory segment, operably linked to said selectable marker coding segment to promote expression of said selectable marker coding segment in said plastids; and

iii) a 3' regulatory segment, operably linked to said selectable marker coding segment to promote stability of mRNA produced during said expression of said selectable marker coding segment in said plastids.

29. A DNA construct as claimed in claim 3, wherein said expressible DNA segment comprises at least one coding segment.

30. A DNA construct as claimed in claim 3, wherein said at least one expressible DNA segment comprises a reporter gene encoding a detectable gene product.

31. A DNA construct as claimed in claim 30, wherein said reporter gene encodes a gene product selected from the group consisting of beta-glucuronidase and luciferase.

32. A multicellular plant stably transformed with the DNA construct of claim 3.

33. A DNA construct as claimed in claim 29, wherein said at least one additional expressible DNA segment contains a selectable marker gene under control of said 3' and 5' regulatory segments.

34. A DNA construct as claimed in claim 29, wherein said selectable marker gene is disposed within said targeting segment operably linked to 5' regulatory segments to enhance gene expression and to 3' regulatory segments to enhance mRNA stability.